

**University of Wyoming
Institutional Animal Care and Use Committee
Standard Operating Procedure for Rodent Genotyping**

Purpose

The purpose of this standard operating procedure (SOP) is to outline the rodent genotyping methods approved by the University of Wyoming IACUC.

Policy

The University IACUC approves several methods of obtaining a source of DNA for genotyping rodents. These methods include ear punch, hair bulb, fecal pellet, buccal swab, and tail biopsy. The least invasive method that will meet the requirements for the research study must be used. The individual that will be implementing the method chosen must be identified in the research protocol and must be trained in the method to be used.

If a researcher would like to use one of the methods outlined in this SOP, the researcher must include the following information in his/her protocol:

1. Reference this SOP and the method that will be used.
2. Identify who will be performing the method as part of the protocol and state that, that individual is qualified to perform the method.
3. If proposing the use of the tail biopsy method, include the age of the animal and if necessary a description of the general anesthetic to be used (see below).
4. If proposing multiple tail biopsies, provide scientific justification and anesthetic to be used (see below).

If a researcher would like to use a method not outlined in this SOP or a variation of a method outlined in this SOP, the researcher must describe the method in detail in the submitted protocol for IACUC approval.

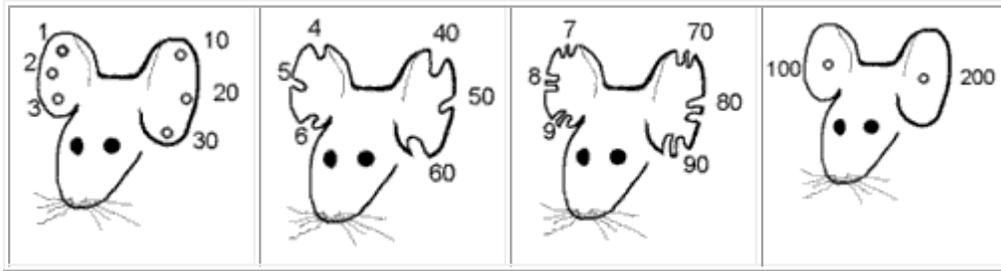
Procedures

The following general requirements apply:

Method	Age	Anesthesia/Analgesia Requirements
Ear Punch	At least 14 days	None required
Hair Bulb	Any age if hair is present	None required
Buccal Swab	Any age	None required
Fecal Pellet	Any age	None required
Tail Biopsy	10-14 days is preferred	Depends on age (see below)

Ear Punching

1. Restrain the mouse by the scruff and using the ear punch, make holes and/or notches in the ears, following an identification chart (see below).
2. Whenever possible, use a simple code to limit the number of notches/punches.
3. Have the identification chart readily available in the animal room to allow prompt identification of individuals.
4. If possible, use the excised tissue as a sample for genotyping, replacing the need for a tail biopsy.



Hair Bulb

1. Scruff the animal.
2. Using forceps, pick a tuft of hair from the ventral side of the animal.
3. Place the hair into a 1.5 ml reaction tube
4. Use a clean pair of forceps for each animal.
5. Return the animal to its cage.

Fecal Pellet

1. Collect fecal pellets from an individual animal using brief gentle manual restraint or by placing it in a clean cage.
2. Place pellet into labeled collection tube.

Buccal Swab

1. Manually restrain the animal by the scruff to maintain its mouth open.
2. Using the swab, vigorously scrape both inner cheeks.
3. Insert cotton swab into labeled collection tube and snip off excess shaft.
4. Return the animal to its cage.

Tail Biopsy (also known as Tail Clipping)

Researchers are required to consider all alternatives to a tail biopsy. Tail biopsies should be used only when the researcher has demonstrated that he/she cannot obtain sufficient amounts of DNA for the specific test being conducted. Obtaining tissue from a mouse for DNA analysis via tail biopsy is a safe, effective and humane procedure when performed properly.

Requirements

1. Tail tip removal should be performed at as young an age as possible.
2. In mice <21 days of age, clipping of the tail can be performed without general anesthesia.

3. In older mice (>21 days of age) general anesthesia is required.
4. Sampling must be performed using sharp, sterile scalpel blades or scissors.
5. Instruments must be disinfected appropriately between animals.
6. The smallest possible sections should be removed (3-5mm is recommended) but no more than one (1) cm may be taken at any age without the use of anesthesia.
7. Repetitive sampling may be approved with total sections removed not exceeding one cm. Repetitive sampling requires the use of anesthesia.
8. Bleeding must be controlled by applying direct pressure to the wound or by the application of heat (cautery,) silver nitrate, or tissue adhesive.
9. The animal should be monitored until it is fully recovered from the procedure/anesthesia.
10. Investigators should be aware that tail ossification rates may be altered in genetically modified mice, and those animals should be monitored for the appearance of pain or distress following the tail snip.
11. If proper procedures are followed, the DNA yield from 5 mm of tail should exceed 50 micrograms, enough for multiple analyses. The DNA yield does not increase proportionally with tail fragment size. If only small amounts of DNA are required, investigators should consider taking only 2 mm of tail.

Sources:

1. University of North Carolina at Charlotte: <http://research.uncc.edu/departments/office-research-compliance-orc/animal-care-use/policies-guidelines-sops> .
2. University of Nebraska Medical Center: <http://www.unmc.edu/iacuc/policies/index.html> .